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Terry Hazen finds, studies and then uses microbes to clean up pollution deep underground, to refine oil before it is pumped and to produce the next generation of green fuels. He catches alligators sometimes, too.

By Bijal Trivedi



Terry Hazen at one of his microbe-feeding wells. (Bijal Trivedi)

A ribbon of blacktop lined with telephone poles is the only human signature for 10 miles beyond the security checkpoint at the <u>Hanford Site</u> in the high plains desert of southeastern Washington. The gently rolling hills are stark, an uninterrupted sprawl of sagebrush and brown cheatgrass, until the harsh geometric silhouettes of entombed nuclear reactors begin to punctuate the landscape. The once prolific nuclear production site has the aura of an Old West ghost town, except for the incongruous presence of bulldozers, trucks and workers in <u>hazmat suits</u>. Today, Hanford is the site of the "world's largest environmental cleanup project."

<u>Terry Hazen</u>, an environmental microbiologist at <u>Lawrence Berkeley National Laboratory</u>, is my guide through this nuclear wasteland. Hazen follows a long line of cleanup specialists who are trying to decontaminate the land, but he has different tools in his arsenal, and they are alive. Hazen plans to cultivate an army of microbial janitors that will use evolved alchemy to convert <u>hexavalent chromium</u> — a carcinogen that, along with the <u>Pacific Gas and Electric Company</u>, played co-villain in the film <u>Erin Brockovich</u> — into chromium III, a benign form of the element.



After 20 miles, we turn left onto a gravel road marked only by one of many generic "Hazardous Area" signs warning of contaminated soil. Within a few hundred yards, we reach a patch of gravel in Hanford's 100-H Area, where Hazen is about to launch an ambitious \$500,000 field experiment.

"I told you it wasn't much to look at," Hazen says almost defensively, gesturing to five metal pipes, each protruding from concrete slabs spaced about 5 feet apart. Unremarkable as they seem, the pipes are actually wells, through which Hazen's team will deliver nutrients deep underground, to trillions and trillions of microbes. The goal is to feed subterranean microbes so they multiply, forming a huge underground treatment zone. When chromium-contaminated water in the aquifer flows through this zone, the microbes will pluck out the toxic chromium VI and convert it to

chromium III, cleansing the water that will then continue to flow into the Columbia River.

Notorious as they may be for doing harm — making us sick and spoiling our food, among other things — microbes play vital roles in the ecosystems where they have evolved over the past few billion years. Hazen recognizes the incalculable skill set of these tiny forms of life and has built his career figuring out how naturally occurring microbes can best serve man: as cleanup crews, as sentinels of human disease, as factory workers for underground oil-refining operations and as producers of the next generation of clean, green fuels.

Although he's seeking out microbes with particular skills, his approach is more than straight bioprospecting. Hazen tries to understand the microbial dramatis personae in an environment, the role each plays and the company it keeps, and how these organisms work together to react to changing temperatures, acidity and chemistry. Such a global or "systems biology" approach is essential to answering pressing scientific questions like how, for example, microbes in the tundra regions of the world will increase the production of methane, a critical greenhouse gas, as permafrost warms. Together, his various microbe-research projects address a swath of A-list environmental issues facing the country.

Hazen's approach at Hanford is based on an idea, popularized by <u>Martin Alexander</u> of Cornell University more than 40 years ago, called the doctrine of infallibility. It contends there is no known compound, man-made or natural, that microorganisms cannot degrade. The trick is to figure out which microbe will degrade what, and when.

Hanford's contamination dates to the 1940s, when the site hosted a frenzied effort to produce plutonium for the <u>Manhattan Project</u>; it was Hanford plutonium that was used in the <u>Fat Man bomb</u> dropped on Nagasaki. The site continued to produce plutonium for the atomic arsenal through the mid-1980s.

The legacy of that plutonium production is a staggering quantity of radioactive and other contaminants buried, stored and discharged into pits or dumped onto the ground, polluting 5 million cubic yards of soil and spoiling 270 billion gallons of groundwater over 80 square miles.

Hazen believes the only practical way to clean up toxic chemicals that escaped beyond the reach of shovels and bulldozers is to encourage the microbes dwelling in the soil and rock to do it for us. Soil of all types teems with life; each gram nurtures anywhere from about 1 million to 10 billion bacteria, representing 4,000 to 10,000 species, each one bringing something different to the table.

Although Hanford ranks as one of the worst, it is just one of more than 70,000 or so dangerously contaminated sites scattered across the United States. Cleanup traditionally involves costly measures like carting dirty soil to distant decontamination plants or entombing toxic material in pits, a process that is often ineffective, Hazen says, because the tombs inevitably leak. The price tag to clean up all these sites is estimated at \$1.7 trillion. If Hazen's Hanford field experiment succeeds, it could reveal a significantly less expensive route to remediate toxic sites nationally and around the world.

In college, Hazen seemed an unlikely candidate to lead the field of environmental microbiology. He attended <u>Michigan State University</u> from 1969 to 1974 and imbibed the culture on which the "party" school rep is based, he says with a grin. At one of those parties, he met his future wife and, he says, "the drinking stopped." He got married, graduated with honors and stayed on a year to snag a master's in parasitology before pursuing his doctorate in parasitology and ecology at <u>Wake Forest University</u>.

Hazen didn't begin his career as an advocate for microbes, or "bugs" as he calls them. Early on, he was interested in how microbes caused disease and how pathogens could survive in different conditions. For his doctorate, he investigated the parasite *Aeromonas hydrophila*, which causes "red sore" disease in fish.

He took his newly minted degree to the <u>University of Puerto Rico</u> in 1979, lured not just by the chance to cruise the Caribbean in his 27-foot sailboat but by the opportunity to explore how microbes, like the bacterium *E. coli*, survive in tropical environments. <u>E. coli</u> was thought to always signal the presence of human waste, but Hazen guessed that wasn't the case in warmer climes.

While in Puerto Rico, Hazen overturned the almost universal assumption that the microbe *E. coli* was always associated with human feces. By collecting water from pristine streams and the leaves of bromeliads in the top of the rainforest canopy, he proved that *E. coli* was indigenous to the Puerto Rican environment. During his eight years on the island, Hazen rose to tenured full professor and chairman of the Department of Biology at the <u>University of Puerto Rico in Rio Piedras</u>, with more than 30 publications within four years — all before he was 30.

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In 1987, Hazen received an offer to move to what is now the <u>Savannah River National Laboratory</u>, at the Department of Energy's 310-square-mile <u>Savannah River Site</u> near Aiken, S.C., where he was hired to study life deep underground, a little-explored field.

At Savannah River, Hazen's interest in microbes grew exponentially; today he is regarded as one of the pioneers of microbial ecology. In the early 1990s, he led the <u>Department of Energy</u>'s first efforts to identify deep subsurface microbes and figure out what they were doing in uncontaminated sediments and groundwater. While drilling in the Savannah River Site, Hazen noticed methanotrophs, or microbes that consume methane, 2,000 feet below — which surprised scientists because the rock there was thought to lack the nutrients to support life. These microbes possessed an almost magical property. They produced a powerful enzyme that could break down more than 300 types of toxic organic contaminants and convert them into carbon dioxide and water.

Hazen reasoned that if he "fed" these methanotrophs, they would multiply, creating swarms of microscopic cleaners that would transform pollutants. All that was needed to encourage these bugs to break down <u>trichloroethylene</u> — the most common organic contaminant, used in dry cleaning, motor degreasing and decaffeination processes — was "a little bit of oxygen and a little bit of methane gas," which he injected into the ground using oil-drilling technology. The approach proved highly successful in field tests — his team cleaned up TCE contamination in a football field-size zone about 300 feet below ground — and the technique became a patented remediation tool.

"What [Hazen's] really known for is pioneering the use of large-scale field studies to understand how key microbes can change soil ecology and do things like remediation," says <u>Adam Arkin</u>, who, with Hazen, co-directs the <u>Virtual Institute for Microbial Stress and Survival</u> at Lawrence Berkeley National Laboratory. "He's almost an engineer, in a sense, because he works on how to use microbes to effect environmental change."

In 1995, the DOE sent Hazen to spearhead the cleanup of an abandoned oil refinery in southern Poland where oil had been dumped in open ponds for more than a century, forming what Hazen called a "sludge lagoon." He showed that cleaning up such sites could be done quickly, to a high standard, just by providing the right mix of air and food to indigenous microbes, which cleaned up the oil-soaked dirt by doing what comes naturally. The project showcased the DOE's expertise and served as the training ground for bioremediation teams all over Europe. "I don't believe in doing the most expensive and the most cool, that sort of thing, unless it is practical," he adds.

When met with an environmental disaster, Hazen doesn't get emotional. He doesn't rant about the extent of the disaster or the impact on the wildlife. Often, he admits, his first impulse is to get a sample. That's exactly what he did when he saw the old sludge lagoon.

When Hazen eventually analyzed the bugs in the Polish sludge, he discovered new acidophiles — microbes that thrive under very acidic conditions and have the rare ability to degrade oil contaminants. Savannah River later patented them.

Hazen began working at the Hanford Site in 2002, when hexavalent chromium concentrations ranged from 100 to 200 parts per billion — 10 to 20 times the allowable limit. The source of the contamination lies about 2 miles west of Hazen's wells, where two reactors operated from the 1940s to the 1960s. Sodium dichromate, used to prevent corrosion, was added to filtered river water and used to cool the reactors. During the 1950s and 1960s, this water was dumped in unlined trenches, which then leaked into the porous soil and aquifer.



Entombed in concrete, these reactors at the Hanford Site will sit in "Interim Safe Storage" for 75 years. (Bijal Trivedi)

The form of chromium known as chromium VI is toxic at high concentrations — with health effects varying depending on the type of exposure; the substance can be absorbed through the skin, ingested or inhaled. The chemical's effects on humans were vividly portrayed in the film *Erin Brockovich*, in which the residents of Hinkley, Calif., were exposed to chromium VI via contaminated drinking water and probably inhalation. The Pacific Gas and Electric Company used chromium VI to prevent corrosion in cooling towers and then dumped the chromium-laced wastewater into unlined ponds during the 1950s and 1960s. Among a laundry list of diseases that Hinkley residents suffered were lung, breast, stomach, kidney and prostate cancer, which are believed to be caused by ingesting chromium VI. The levels that Hazen sees at his wells can cause deformities in or kill salmon fry.

Hazen's team began feeding the Hanford microbes in 2004. They injected 40 pounds of nutrients — a viscous, honey-like substance — into the wells and a few days later began taking samples of water and soil to be analyzed in Hazen's Berkeley lab. Within three weeks, it was clear that the microbes were enjoying the new diet and thriving; the population boomed 1,000-fold and "the chromium levels were undetectable," Hazen says, holding back a triumphant smile. "It lasted for almost four years."

Unlike other microbiologists, Hazen isn't interested in studying bacteria one species at a time in the lab. He wants to understand how they work together in the real world to perform complex chemistry that would be tremendously impractical and expensive for humans to mimic.

In 2008, after the hexavalent chromium levels rose again to 100 parts per billion, Hazen's team fed the microbes a second meal of about 10 pounds of nutrients. Within 24 hours the odor of hydrogen.sulfide— rotten egg gas — began wafting out of the wells. It's the "sweet smell of success," says Hazen with a smile, an indication that the toxic chromium was now being downgraded into the relatively harmless form that will cling to sediments and stay out of water — and out of the Columbia River, an important breeding ground for salmon. Hazen notes that there seems to be some type of "memory response" that occurs when the microbes are fed a second time, triggering a faster reaction.

In March, Hazen's team began the largest chromium bioremediation effort at Hanford to date by shooting 55 gallons of lactate into one of the wells. Lactate — a compound similar to the one that builds up in your muscles when you exercise — is an environmentally friendly food that allows bacteria to grow and multiply. The team will pump the liquid 40 feet down into the aquifer where it will disperse over an area about 50 by 100 feet — creating a treatment zone.

Hazen's approach exploits a fundamental mechanism of metabolism. When humans digest food, they take electrons from the foods they eat and pass them off to the oxygen they breathe in. Similarly, microbes in the soil metabolize the nutrients that come through the wells, strip off some of the electrons and, through a series of chemical reactions, transfer the electrons to an electron acceptor. But different microbes have evolved to breathe different substances. Some microbes breathe oxygen, some nitrate, some carbon dioxide, and others breathe chromium.

Once the oxygen in the soil runs out, the microbes that breathe nitrate thrive. Hanford soil is rich in nitrate because it was used to process nuclear material and then dumped. Once nitrate runs low, then chromium-breathers dominate. These microbes will use the lactate and channel their electrons to chromium VI, converting it to chromium III (which is actually an essential element in the human diet, with a recommended daily intake). When the chromium VI runs out, which is Hazen's goal, then microbes breathing iron, sulfate and carbon dioxide dominate, sequentially converting these substances into iron II, hydrogen sulfide (the rotten egg gas) and methane. So when Hazen smells rotten egg gas, he knows that the chromium has been modified.

Chromium III doesn't disappear. But rather than leaching into the water and flowing into the river, it sticks to the soil. Hazen says the site will need perpetual monitoring as shifting environmental conditions — in particular the oxygen and nitrate levels — could kick the chromium back to its more dangerous state.

Manipulating a cast of thousands of different types of bacteria to perform a set task is a huge leap forward in bioremediation. It is only in the last 10 to 15 years that scientists have had the technology to even identify the thousands of species of microbes living down there. "Giving the microbes the right food to do the job — he's been one of the masters at making that happen," Arkin says.

Long-term, difficult experiments like the one at Hanford are what compels Terry Hazen, says <u>Judy Wall</u>, a biochemist at the University of Missouri and a collaborator. "Terry can anticipate the trajectory of an experiment, ask long-term questions and collect a range of data that can still be mined years down the road with new technology," she says. "That really takes great insight and long-term vision."

Hazen anticipates that the microbial-remediation approach used at Hanford will become increasingly important. Studies in the last decade have shown that even ultra-low concentrations of many contaminants — like petroleum products — can disrupt the reproductive systems of humans and other animals, suggesting that many sites will need to meet a higher standard of cleanup. The only way to reach safe levels may involve the use of microbes. "Methanotrophs can get contaminants to parts per trillion and basically degrade until there is nothing there," Hazen says.

In 1998, Hazen was lured to Lawrence Berkeley National Laboratory, a renowned center for biotechnology research. There, he now runs an empire with many projects and hundreds of collaborators; he leads the ecology department, the environmental biotechnology center and the microbial communities department of the <u>Joint BioEnergy Institute</u>, to name just a few of his efforts. All of his projects are somehow tied to remediation.

While an adamant environmentalist, Hazen is unapologetic about working both ends of the energy equation, using microbes not just for cleanup, but to improve oil extraction and refining, and to produce biofuels. "We can't say, 'Oh we don't want to work on oil because of greenhouse gas and all this crap," Hazen says softly but with a tinge of impatience. "We're going to need oil and some of those products anyway. So why not change it to something that is less toxic to the environment?"

Hazen says that he knew all along that microbes could be used for in situ oil refining. But, he says, "Until we hit this energy crisis here recently, nobody was interested in it." When oil prices spiked, interest grew; in 2007, the University of California, Berkeley, received \$500 million from <u>BP</u> to set up the <u>Energy Biosciences Institute</u>. Before becoming Secretary of Energy, <u>Steven Chu</u>, then the director of the Lawrence Berkeley National Laboratory, tapped Hazen to lead the search for oil-refining microbes.

"Terry was an easy choice to run the Microbially Enhanced Hydrocarbon Recovery program because he is both an excellent scientist and a strong manager," Chu said in e-mail interview. "He has been a leader in microbial environmental biology and ecology, and the results of his research [are] now being applied in the bioremediation of many contaminated sites."

In the petroleum reservoirs of Alaska, the Gulf of Mexico and Colorado, Hazen has launched projects seeking out microbes that can make oil easier to extract by making it less viscous or by converting it to hydrogen or methane gas. Hazen says that if microbes could perform such chemical processing underground, it would eliminate, or at least reduce, the environmental damage and pollution caused by oil refineries, the use of chemicals to fracture underground formations and other methods of extracting oil and gas from underground reservoirs.

Hazen is confident he'll hit pay dirt. In 2008, he co-authored a paper announcing the discovery of a new bacterium that resides 2.8 kilometers down a South African goldmine. Hazen says that microbes like this extremophile, which can survive a hot, salty environment, could provide clues to how microbes could survive oil reservoirs. "I suspect that we'll find even that same exact bug in some of these oil wells, too," he says.

'O K, a story," Hazen says abruptly. "Have I told you about catching alligators?"

Hazen doesn't particularly look like an outdoorsman. He's tall — about 6 feet 5 inches — with a heavy physique. Bespectacled, ruddy cheeked, with an almost grandfatherly demeanor, Hazen speaks softly, his voice often trailing off at the end of his sentence. But all that changes when he tells the story of the alligators that were dying in South Carolina's Par Pond from 1976 through 1977. Rumors hinted that warm water released from a nuclear reactor on the pond was to blame. Hazen quickly brushed up on the problem, wrote a proposal on a Friday and sent it off to the Department of Energy. By Monday, he had funding and a permit to catch alligators.





Terry Hazen and his alligator in the 1970s. (Courtesy of Terry Hazen)
Leaning forward, he describes paddling though the steamy swamps of <u>Green Pond, S.C.</u>, on a moonless June night, searching for alligators while dodging water moccasins falling from trees. With the help of a postdoctoral researcher, Hazen would haul a thrashing alligator into the boat, mouth gaping, and "quickly put a rubber band around it."

After much hard work on samples drawn from the captured gators, Hazen proved that a microbe was killing them. But the warmer waters from the reactor's cooling system had played a role, creating cozier conditions for the microbe. Hazen clearly cherishes the alligator adventure; a picture on his Web site's home page shows him sitting next to a gator, holding its mouth open.

Hazen's latest adventure returns him to the warm and wet clime of his old stomping grounds in the Puerto Rican rainforests, where he's looking for microbes that degrade cellulose and lignin, tough structural components of plants that are currently foiling attempts to produce ethanol from them. From roaming these forests more than 20 years ago, Hazen knows that in the lush rainforest, organic matter disappears fast and that microbes are responsible. He reasons that the chances of finding new organisms and new enzymes for degrading plant material would be "astronomically greater" in such a place.

To attract the "right" type of microbe — one that efficiently breaks down cellulose, which can be converted to ethanol that can be used as fuel — Hazen's team filled 400 little mesh bags with switchgrass, a tough native grass that can grow to 10 feet tall and dominated the American prairie a century ago. The bags were then buried in various locations in the rainforest. Switchgrass is considered a better choice for biofuels production than corn because the net energy output is more than 20 times higher.

By analyzing the DNA of microbes munching on the switchgrass, Hazen can get a rough idea of which microbe families are present and which genes are at work. Because genes that make cellulases "have a characteristic look," Hazen's team can fish out promising DNA sequences from the microbes and use them to reconstruct enzymes that might break down cellulose; they are then tested in the Berkeley lab. This past summer, his team made a breakthrough. "Two of them look like they are golden — like they really degrade things fast and are much better than a lot of things that we've seen," Hazen says.

Back at Hanford, Hazen's microbial army is preparing for an epic cleanup. As bone-chilling winds stiffen our faces and hands at the Hanford Site, the microbes await peacefully 40 feet below at a constant 53 degrees Fahrenheit. If all goes according to plan, Hazen's generous food delivery in March will kick the microbes into high gear, triggering a growth spurt and population boom, particularly of the chromium-breathing microbes that will transform a toxic chemical to a beneficial form.

But Hazen isn't one to sit back and marvel at the bugs and the chemical stunts they perform. Like a boss who expects the most from his employees, he explains that they are just doing what they have evolved to do. "It's more surprising," he says, "when they don't do what I expect them to do."